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PRINCIAL INVESTIGATOR: Yiliang Liu, Ph.D.

CONTRACTING ORGANIZATION: Long Island Jewish Medical Center
New Hyde Park, New York 11040

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6. AUTHOR(S)
Yiliang Liu, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Long Island Jewish Medical Center
New Hyde Park, New York 11040

E-MAIL:
liu@lij.edu

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SYNUCLEIN γ (SNCG) AND BREAST CANCER PROGRESSION.

We recently identified and cloned a novel breast cancer-specific gene BCSG1 by direct differential cDNA sequencing. BCSG1 has a great sequence homology with Alzheimer disease (AD)-related neural protein synuclein, and thus was also named as synuclein γ (SNCG). We demonstrated that: 1) SNCG expression was a stage-specific in human breast: undetectable in normal or benign breast lesions, low level and partial expression in low grade ductal carcinoma *in situ* but extremely high level in advanced infiltrating breast cancer; 2) SNCG expression in human breast cancer cells is dramatically suppressed by tumor growth inhibitor oncostatin M (OM), a cytokine predominantly produced by activated T cells and macrophages; 3) overexpression of SNCG in breast cancer cells led to a significant increase in cell motility and invasiveness *in vitro* and a profound augmentation of metastasis *in vivo*. Our data suggest that the member of neural protein synucleins might have important functions outside the central nervous system and play a role in breast cancer progression.

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I. BACKGROUND AND SIGNIFICANCE

Metastasis is proposed to depend on five major activities: angiogenesis, cellular attachment, proteolysis, migration through the barrier into the secondary sites, and, of course, colonization and proliferation in the distant organs. We have recently identified and cloned a putative breast cancer specific gene, BCSG1, which was **(a)** highly expressed in mammary gland relative to other organs and was **(b)** high abundance in a breast cancer cDNA library but scarcely in a normal breast cDNA library (1). We have demonstrated that expression of BCSG1 correlate with clinical aggressiveness and may indicate breast cancer malignant progression leading to metastasis. We also provided evidences linking overexpression of BCSG1 in human breast cancer cells with increased migratory motility and invasive activity *in vitro* and a profound augmentation of metastasis *in vivo* (2). The use of BCSG1 gene could be of importance in differentiating atypical proliferative breast lesions or noninvasive carcinoma *in situ* from malignant and invasive cancer and may be useful in screening of breast biopsies for potential abnormalities. In addition, if overexpression provides a therapeutic target, then BCSG1 may be useful in clinical management and treatment of breast cancer.

Interestingly, BCSG1 revealed no homology to any other known tumor metastasis related factors; rather, BCSG1 revealed extensive sequence homology to with Alzheimer disease (AD)-related neurotic proteins synuclein α (SNCA) and synuclein β (SNCB) that are mainly localized to brain (3-9). Therefore, BCSG1 was also named as synuclein γ (SNCG). The pathological hallmark of AD is amyloid deposition in neurotic plaques and blood vessels. The major constituent of amyloid is a 39-43 AA peptide named Aß component and SNCA is the second intrinsic constituent of amyloid. An elucidation of the reasons for SNCG overexpression in infiltrating breast cancer and SNCG-induced metastasis may shed some light on the pathogenesis of not only breast cancer progression but also neurodegenerative disorders.

II. WORK ACCOMPLISHED

The overall hypothesis to be evaluated is that up-regulation of BCSG1/SNCG expression may indicate breast cancer malignant progression from a benign breast or a low grade *in situ* carcinoma and to a highly infiltrating carcinoma. The overexpression of BCSG1 may correlate with clinical aggressiveness of breast cancers. Therefore an alternations of BCSG1 expression may lead to an abnormal growth and malignant progression.

Overexpression of SNCG in breast cancer cells led to a significant increase in motility and invasiveness *in vitro* and a profound augmentation metastasis *in vivo* (2). This is the first report indicating the potential involvement of synuclein in the non-neurotic disease. An elucidation of the reasons for SNCG overexpression in infiltrating breast cancer and SNCG-induced metastasis may shed some light on the pathogenesis of not only breast cancer progression but also neurodegenerative disorders. Please see attached paper for detail description.

Previously, we have shown that synuclein γ (SNCG), a member of the brain protein synuclein family, is highly expressed in human infiltrating breast carcinomas but not expressed in normal or benign breast tissues. The SNCG mRNA was also detected in several human breast cancer cell lines with the highest expression found in H3922, a cell line derived from an infiltrating ductal carcinoma. In this study, we show that expression of SNCG mRNA in H3922 cells is significantly decreased by treating cells with the cytokine oncostatin M (OM) who has a growth-inhibitory effect on these cells (10). A decrease in SNCG mRNA level can be detected as early as 30 min after OM addition. By 4 h OM treatment, the level of SNCG mRNA was decreased to 70%

of control, and by 24 h the mRNA was below undetectable level. Since OM-induced growth inhibition occurs after 2 to 3-days, the down-regulation of SNCG expression appears to proceed the effect of OM on cell growth. Additional experiments to measure the transcriptional rates of SNCG indicate that the observed OM-induced down-regulation of SNCG mRNA occurs mainly at the transcriptional level. In an attempt to examine the role of SNCG gene in the proliferation of breast cancer cells, SNCG cDNA was stably transfected into MCF-7 cells that do not express endogenous SNCG gene. Examination of cell growth under anchorage-dependent and anchorage-independent conditions demonstrates that over expression of SNCG gene significantly stimulated the growth of MCF-7 cells both in monolayer culture and in soft agar. These data together suggest that SNCG may be one of the contributing factors that promote the uncontrolled growth of malignant mammary cells.

III. TRAINING

This is PI's first independent grant. The proposed studies of the current grant application includes a variety of different aims and experiments ranging from basic molecular biology, cell biology, in vivo orthotopic nude mice model for tumor growth and metastasis, and a clinical oriented study on screening clinical human breast specimens. This is the first time that PI has a chance to independently carry out a very challenge, yet ambitious, multi display project. During the last year, PI has gained a lot of experience on animal model and in vivo analysis of tumor metastasis. The success on the current grant proposal will encourage and facilitate PI's future career development as an independent clinically oriented breast cancer investigator.

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